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The Effects of Aggregation and Microhabitat on Desiccation and Body Temperature of the Black Turban Snail, *Tegula funebralis* (A. Adams, 1855)

by

KAREN E. MARCHETTI

University of California, Bodega Marine Laboratory, Box 247, Bodega Bay, California 94923, U.S.A.

AND

JONATHAN B. GELLER[1]

Bodega Marine Laboratory[2], Box 247, Bodega Bay, California 94923, U.S.A. and
Department of Zoology, University of California, Berkeley, California 94720, U.S.A.

Abstract. We studied the roles of aggregative behavior and microhabitat differences in reducing desiccation of the rocky intertidal prosobranch gastropod *Tegula funebralis* (A. Adams, 1855) at Bodega Bay, California. Solute concentration of extra-corporeal water (ECW) was measured as an indication of desiccation through evaporative water loss. In the majority of field collected samples, aggregated *Tegula funebralis* had lower ECW solute concentrations than solitary individuals. In laboratory experiments, smaller individuals desiccated faster. In the field, microhabitats had a large influence on desiccation stress: both aggregated and solitary individuals in protected microhabitats during times of exposure had lower solute concentrations than individuals in exposed microhabitats. Microhabitat differences had no effect on body temperature, but aggregated snails were significantly cooler.

INTRODUCTION

Organisms inhabiting the rocky intertidal zone are continuously subject to a variety of physical stresses. During each tidal cycle, these organisms may be subject to conditions of essentially a terrestrial environment (Davies, 1969; Vernberg & Vernberg, 1972). Evaporative water loss upon exposure to wind and solar radiation may result in high levels of desiccation. Behavioral and physiological adaptations to desiccation are therefore important for survival in the rocky intertidal environment (Garrity, 1984).

The purpose of this study was to investigate the effects of aggregative behavior on desiccation and body temperature in *Tegula funebralis* (A. Adams, 1855), and some of the factors influencing this behavior. *Tegula funebralis,* the black turban snail, is a common middle intertidal gastropod occurring from Vancouver Island, British Columbia, to Central Baja California (Morris *et al.,* 1980). Although its spatial distribution has been studied (see, for example, Wara & Wright, 1964; Paine, 1969; Fawcett, 1984), little attention has been given to the aggregative behavior of this species and its possible role in reducing desiccation stress.

We examined this question by comparing extra-corporeal water (ECW) samples from aggregated and solitary snails. Extra-corporeal water, held within the mantle cavity, is a source of evaporative water loss in exposed snails. Wolcott (1973) showed that, for limpets, mortality during low tide exposure is associated with concentration of internal fluids resulting from evaporative water loss.

Microhabitat choice by snails may also play an important role in avoiding desiccation (Garrity, 1984). It was therefore our further intent to investigate the relative importance of aggregation and microhabitat selection in reducing desiccation stress.

[1] Author for correspondence.
[2] Address for correspondence.

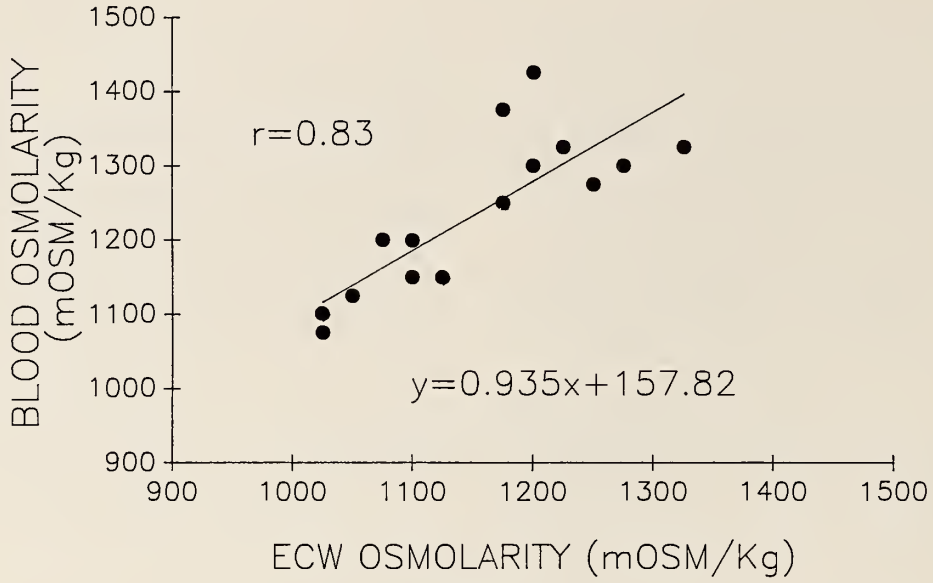

Figure 1

Relationship between blood osmolarity and extra-corporeal water osmolarity in *Tegula funebralis*. The plotted line and equation are the linear regression of these variables.

MATERIALS AND METHODS

This study was conducted during April and May 1986, on the Bodega Marine Life Reserve, Sonoma County, California. Laboratory studies were conducted at the Bodega Marine Laboratory. Field data were collected during low tides, at a tidal level of approximately 1.5 m above mean lower low water. Temperature, wind conditions, and time of day differed during collection of field data, but conditions were qualitatively similar. The substratum was heterogeneous, consisting of bare flat rock, crevices, and various degrees of algal cover (mainly *Endocladia muricata* [Postels and Ruprecht, 1840], *Pelvetiopsis limitata* Gardner, 1910, and *Porphyra* sp.). SUTHERLAND (1970) and WOLCOTT (1973) provide further details of the study site.

Field Studies

To use ECW as a measure of desiccation, it was first necessary to determine that *Tegula funebralis* is isoosmotic with its aqueous environment, and that osmolarity of internal fluids increases with increasing osmolarity of ECW. To confirm this relationship, we collected snails from the field and placed them in the sun for various lengths of time. This allowed testing over a wide range of evaporative water loss. Extra-corporeal water samples were collected by pressing lightly on the operculum with a capillary tube (0.9 mm diameter), allowing a small amount of ECW to be drawn into the tube as the snail withdraws and ECW is forced out of the mantle cavity. Capillary tubes were immediately plugged with plasticene clay, to prevent further evaporation of samples.

Blood samples were taken by removing individuals from their shells, pat-drying any residual ECW, and cutting the foot with a razor blade. A blood sample large enough for analysis could then be drawn by placing a small capillary tube (0.5 mm diameter) into the laceration and applying pressure to the foot. For each snail, 8 μL samples of both blood and ECW were analyzed using a Wescor 5130 C vapor pressure osmometer. Solute concentration (osmolarity) was measured in milli-osmoles per kilogram (mOsm/kg) blood or ECW.

Extra-corporeal water was sampled in the field in order to compare differences in extent of desiccation between aggregated and solitary *Tegula funebralis*. Sampled aggregations always consisted of at least 10 individuals, each in contact with at least one other snail. In the first trials, solitary individuals sampled were always within 30 cm of a given sampled aggregation, and at least 5 cm away from any other snail, following the criteria of GALLIEN (1985). This procedure assured that aggregated snails and surrounding solitary snails were from the same microhabitat. Seven sets of aggregations and nearby solitary snails were sampled. As a measure of size, the greatest shell width was recorded for each individual.

A second set of field samples tested the effect of microhabitat on level of desiccation. Extra-corporeal water samples were taken from snails within two distinct microhabitats: exposed (bare rock with no crevices) and protected (containing crevices and[or] algal cover). Temperatures of all individuals in the second field sampling were also measured using a Keithly 870 K-type thermocouple thermometer. The probe was placed against the foot, and the snail was allowed to retract into its shell, pulling the probe into the mantle cavity. Snails were in contact with only the

Table 1

Two-way analysis of variance testing the effects of different field sites and aggregation on extra-corporeal water solute concentration in *Tegula funebralis*. (Significance levels: ** $P < 0.01$; *** $P < 0.001$.)

Source	SS	DF	MS	*F*-ratio
Site	59,640.2	6	9940.0	3.2**
Aggregation	92,685.1	1	92,685.1	29.2***
Site × aggregation	64,878.2	6	10,813.0	3.4**

probe for the duration of the reading, with minimal contact with fingers which may warm the snail.

Laboratory Studies

The effect of aggregative behavior on decreasing desiccation stress was also examined in the laboratory. Artificial aggregations consisted of seven snails, five of which were randomly chosen to be sampled for ECW. Both solitary snails and artificial aggregations of small (9–12 mm) and medium sized (16–18 mm) snails were placed in 4-cm diameter cells in a plastic tray while being desiccated. Owing to their size, solitary snails and artificial aggregations of large (22–25 mm) snails were placed in larger 4.5 × 5.5-cm rectangular cells while desiccating. Snails were restrained in cells by a cover of 10-mm mesh nylon netting. Snails were desiccated under two lightbulbs (75 and 100 W) situated 35 cm almost vertically above them. Air was moved over the snails by placing a household electric fan 65 cm vertically above them. Ten aggregated individuals and 10 solitary individuals were sampled every hour for six hours. Measures of osmolarity and temperatures were recorded as an indication of level of desiccation stress. A different set of snails was used for each period of time.

Stress resulting from desiccation was assayed in a second group of snails (all 16–18 mm; desiccated for 4, 6, and 8 hr) by measuring the amount of time necessary for the individual to fully emerge from its shell, adhere to the container, and resume function of cephalic tentacles upon rehydration in normal seawater. For brevity, this behavior is henceforth referred to as emergence. Osmolarity of ECW was sampled before testing for time of emergence: these values were used as a further measure of desiccation.

Behavioral Tests

We examined two potential cues that may be involved in the aggregative behavior of *Tegula funebralis*. First, in order to determine possible preference for light or dark substrata, eight snails were placed uniformly (one per square; see below) in a 19 × 30 × 4-cm pan containing a checkerboard pattern of alternating black and white 9 × 5-cm squares. Locations of snails were noted after 30 min

Table 2

Analyses of covariance (snail size as covariate) testing the effects of aggregation and snail size on desiccation of *Tegula funebralis* as measured by solute concentration of extra-corporeal water. Snails were measured in seven sites on two consecutive days. Differences in slope for aggregated and solitary snails were tested before ANCOVA was performed: slopes within sites were not different except for site 6. (Significance levels: * $P < 0.05$; ** $P < 0.01$; ns, $P > 0.05$.)

Source	SS	DF	MS	*F*-ratio
Site 1				
Aggregation	1396.3	1	1396.3	0.55ns
Size	2160.7	1	2160.7	0.86ns
Residual	68,005.9	27	2518.7	
Site 2				
Aggregation	52,285.0	1	52,285.0	6.14*
Size	9707.7	1	9707.7	1.14ns
Residual	136,293.9	16	8518.4	
Site 3				
Aggregation	2889.4	1	2889.4	5.67*
Size	108.7	1	108.7	0.21ns
Residual	11,210.2	22	509.6	
Site 4				
Aggregation	7093.9	1	7093.9	2.36ns
Size	11,762.1	1	11,762.1	3.91ns
Residual	51,112.9	17	3006.6	
Site 5				
Aggregation	14,858.6	1	14,858.6	8.84**
Size	648.6	1	648.6	0.39ns
Residual	36,995.6	22	1681.6	
Site 6				
Aggregation	41,234.5	1	41,234.5	23.4***
Size	27,555.8	1	27,555.8	15.6**
Aggregation × size	23,625.8	1	23,625.8	13.4**
Residual	38,746.9	22	1761.2	
Site 7				
Aggregation	16,576.2	1	6576.2	6.6*
Size	8825.9	1	8825.9	3.5ns

(little movement was observed after this period). Twenty trials of this test were performed, and each individual snail was tested only once.

Secondly, we examined the role of mucus trail following in the formation of aggregations. The following experiment tested whether snails will choose to follow a conspecific mucus trail regardless of substrate color, or move to dark substrata regardless of the presence or absence of a mucus trail. Mucus trails were created by allowing one snail to move freely on a 19-cm diameter clear plastic sheet placed in a 19-cm petri dish divided into four equal-sized alternating black and white quadrants. This plastic disk could then be rotated such that the trail led to a white or a black

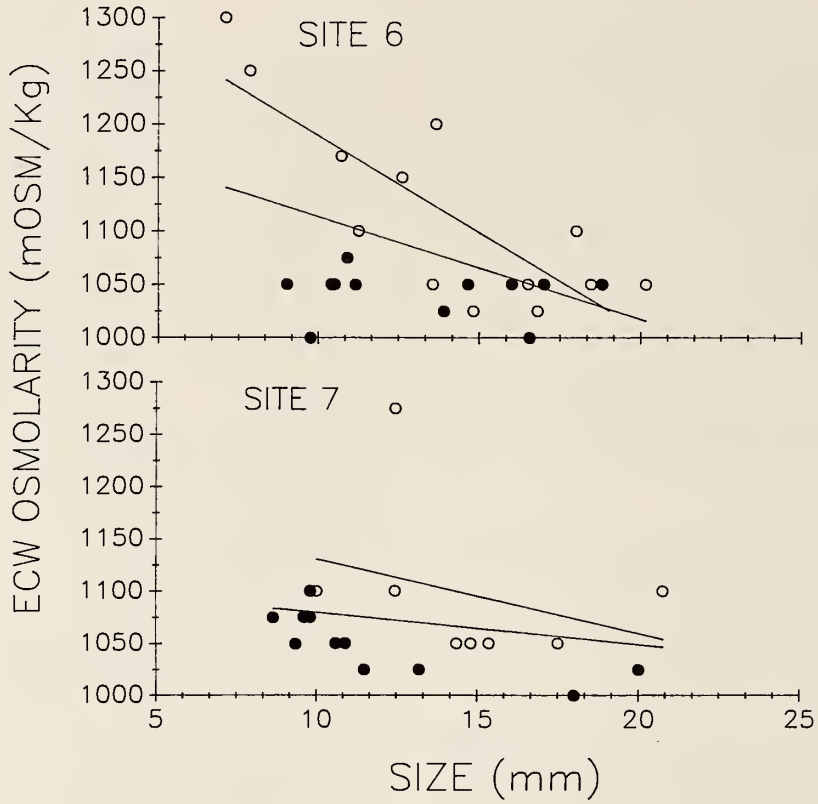

Figure 2

Relationship between snail size and osmolarity of extra-corporeal water of aggregated snails (solid circles and bottom line) and solitary snails (open circles and top line) snails. In site 6 (top graph), slopes are significantly different; in site 7 (bottom graph), slopes are not different but solitary snails are significantly more desiccated (see Table 2).

quadrant. For each mucus trail, a second snail was tested with the trail leading to a dark quadrant, and a third snail was tested with the trail leading to a white quadrant. This procedure was repeated 24 times.

RESULTS

Field Studies

The validity of extra-corporeal water as an indirect measure of blood concentration, and therefore desiccation, was confirmed (Figure 1). Results of a linear regression revealed that solute concentration (Y) of blood increased with that of ECW (X) ($r = 0.83$; $Y = 0.935X + 157.82$; $n = 18$, $P < 0.001$).

A preliminary two-way analysis of variance (ANOVA) of the results from the first field samples indicated significant differences in solute concentration among the seven sites and between solitary and aggregated individuals (Table 1). For easier interpretation, separate analyses of covariance (ANCOVA) were performed for each site, with snail size as the covariate. The results of these analyses are presented in Table 2, and representative data are shown in Figure 2. In two of the seven sites (sites 1 and 4), there were no significant differences in ECW solute concentration between aggregated and solitary snails, nor any relationship to snail size. In four of the seven sites (sites 2, 3, 5, 7), ECW solute concentration differed significantly between aggregated and solitary snails, but again had no relationship to snail size. In one site (site 6), the slopes of the regression lines for aggregated and solitary snails differed: inspection of the data (Figure 2) indicates that in this site, differences in osmolarity between aggregated and solitary snails vary with snail size, with solitary snails more desiccated and this difference most pronounced for small snails. It should be noted that in all seven sites, the slopes of regression lines relating snail size to ECW solute concentration were always negative, but significantly so only in site 6.

The results of the second field trials, which considered microhabitat as well as aggregation, indicated that the effect of microhabitat on desiccation is also important (Figure 3). A two-way ANOVA showed that the effect of microhabitat (protected vs. exposed) on ECW concentration was highly significant ($F_{1,93} = 33.40$; $P < 0.001$),

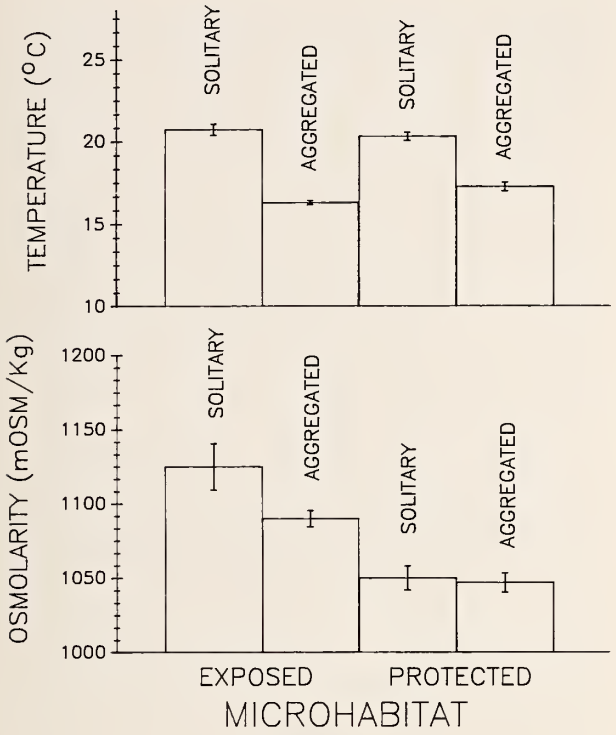

Figure 3

Temperatures (top graph) and extra-corporeal water osmolarity (bottom graph) of solitary and aggregated *Tegula funebralis* in protected (crevices or algal cover) and exposed (open surfaces) microhabitats. Temperature did not differ between microhabitats, but aggregated snails were significantly cooler. Osmolarity was lower in protected microhabitats, but solitary and aggregated snails did not differ.

while in this case the effects of aggregation and the interaction between aggregation and microhabitat were not significant (aggregation: $F_{1,93} = 3.29$; $0.08 > P < 0.05$; interaction: $F_{1,93} = 2.60$; $P > 0.1$). In these trials, both aggregated and solitary snails in protected habitats sustained lower levels of evaporative water loss than did aggregated or solitary snails in exposed microhabitats: mean values (±SD) for protected microhabitats were 1048 ± 38 mOsm/kg ($n = 24$) and 1050 ± 40 mOsm/kg ($n = 25$) for aggregated and solitary snails, respectively; for exposed microhabitats, mean values were 1090 ± 26 mOsm/kg ($n = 23$) for aggregated snails and 1125 ± 78 mOsm/kg ($n = 25$) for solitary snails (Figure 3).

Protective cover had no significant effect on snail body temperature (Figure 3). A two-way ANOVA showed no significant effect of microhabitat (exposed vs. protected) ($F_{1,93} = 1.26$; $P > 0.26$), while aggregated snails were significantly cooler than solitary snails ($F_{1,93} = 229.67$; $P < 0.001$). There was also a significant interaction between aggregation and microhabitat ($F_{1,93} = 11.51$; $P < 0.01$): inspection of the data indicates that the difference between aggregated and solitary snails is greatest in exposed microhabitats (Figure 3). Mean body temperatures (±SD) for aggregated snails were 17.2 ± 1.0°C in protected microhabitats and 16.3 ± 0.5°C in exposed microhabitats. For solitary snails, mean body temperatures were 20.3 ± 1.3°C (protected) and 20.7 ± 1.7°C (exposed). In addition to reducing evaporative water loss, as shown above, aggregative behavior may be a means of reducing body temperature.

Table 3

Two-way analysis of covariance (time of exposure as covariate) testing the effects of time of exposure, snail size, and aggregation on desiccation of *Tegula funebralis* as measured by solute concentration of extra-corporeal water. (Significance levels: * $P < 0.05$; *** $P < 0.001$; ns, $P > 0.05$.)

Source	SS	DF	MS	*F*-ratio
Aggregation	52,586.5	1	52,586.5	3.46ns
Snail size	103,725.6	2	51,862.8	3.41*
Time of exposure	4,428,927.7	1	4,428,927.7	291.48***
Aggregation × snail size	20,184.6	2	10,092.3	0.66ns
Aggregation × time	39,440.5	1	39,440.5	2.60ns
Snail size × time	527,567.2	2	263,783.6	17.36***
Aggregation × snail size × time	33,544.3	2	16,772.1	1.10ns
Residual	4,862,293.4	320	15,194.7	

Laboratory Studies

Results from the laboratory agreed with those from the field, further supporting the hypothesis that aggregative behavior reduces desiccation stress. For all size classes combined over all time periods (1–6 h), solitary snails had higher osmolarity of ECW than aggregated snails ($F_{1,264} = 19.49$; $P < 0.001$). A more detailed two-way ANCOVA (Table 3) (time as the covariate) indicates significant effects of time and snail size on ECW concentration ($P < 0.05$) and a near significant effect of aggregation ($P = 0.064$): under laboratory conditions, time of exposure appeared to have the greatest effect on desiccation. The only significant interaction in the three-way ANOVA was that of time and snail size (Table 3). This can be interpreted as a proportionally greater effect of time of exposure on small snails than on large snails.

Desiccation affected time for emergence: two separate one-way ANCOVAs, with aggregation as the main factor and time of exposure or final osmolarity as the covariate, showed that both time of exposure and osmolarity of ECW had significant effects on time for snails to emerge from their shells following rehydration (time: $F_{1,93} = 43.85$; $P < 0.001$; osmolarity: $F_{1,93} = 53.75$; $P < 0.001$). In neither case did aggregation significantly effect time for emergence

($0.09 > P > 0.05$), nor were there significant interaction terms.

Behavioral Tests

When snails were placed on a substratum with a checkerboard pattern of black and white squares, six or more of the eight snails were found to situate themselves on black squares in 18 of the 20 trials performed. Using this conservative criterion for preference (that is, six or more of eight snails), a chi-square test indicates that this result is significantly different from an null hypothesis of even frequency of preference for black or white ($\chi^2 = 12.8$, df = 1, $P < 0.001$).

In the experiment testing the role of mucus trails, snails placed on a mucus trail leading to a black quadrant went to a black quadrant 21 times, while 3 snails went to a white quadrant. This differs significantly from a random distribution ($\chi^2 = 13.5$, df = 1, $P < 0.001$). This first test, however, does not differentiate between preference for a black substratum vs. trail following. In the second test, most (21 of 24) of the disks had one or two mucus trails (depending on the path taken by individual snails) leading to a black quadrant. When the disk is rotated one quarter turn, these trails lead to white quadrants. If mucus trail following is of primary importance, the null hypothesis is that all of the snails should have followed a trail to a white quadrant. Instead, all 24 snails went to a black quadrant, and a chi-square test (confined to these 21 snails) rejects this null hypothesis ($\chi^2 = 21$, df = 1, $P < 0.001$).

DISCUSSION

This study suggests that aggregative behavior in *Tegula funebralis* may be of importance in reducing desiccation stress. Aggregated snails in the laboratory and in the field were shown to lose less water after a given period of time desiccated than solitary snails. In addition, although ECW concentration and not aggregation per se significantly affected the time taken for emergence, aggregated snails under field conditions should resume normal activity more rapidly than solitary snails owing to the correlation between aggregation and extent of desiccation. Previous studies on different organisms support the conclusion that aggregative behavior reduces desiccation stress. Warburg (1968) showed that aggregated terrestrial isopods lost water at one-half the rate of solitary individuals owing to a reduction in exposed surface area-to-volume ratio. Snyder-Conn (1980) demonstrated enhanced survivorship of aggregated hermit crabs under desiccating conditions. Formation of aggregations reduces water loss and enhances survivorship in a tropical neritid (Garrity, 1984; Garrity & Levings, 1984). Many studies have indicated that desiccation rates increase with decreased body size (see, for example, Davies, 1970; Wolcott, 1973). Our observations agree with these findings: small snails desiccated significantly faster than large snails regardless of aggregated or solitary conditions.

Aggregation may also be a means of regulating body temperature, and therefore metabolic rate, while exposed. In many invertebrates, metabolic rate varies with changes in salinity and(or) temperature. The ability to withstand a short-term rise in ambient temperature or salinity without a significant rise in metabolic rate may be essential to the maintenance of energetic gain in organisms that experience variable environmental temperatures and reduced food availability upon exposure (Newell, 1979). Aggregated *Tegula funebralis* in different microhabitats (protected or exposed) had similar temperatures, and these temperatures were lower than those of solitary snails in either microhabitat. This may be due to water held between individuals, thereby keeping snails at a more constant and lower temperature.

It is of interest to determine whether aggregative behavior functions primarily as a means of reducing desiccation, or whether aggregations are formed as a result of snails converging into protected areas upon exposure. Several observations from this study suggest that aggregations in protected microhabitats may be formed artifactually, owing to crowding into protected areas, while those in exposed microhabitats may be formed actively, owing to orientation toward other snails. First, differences in desiccation stress (due to evaporative water loss) were found to be largest between aggregated and solitary snails in exposed microhabitats, but were not found to exist between aggregated and solitary snails in protected microhabitats. That is, desiccation stress in solitary individuals in exposed microhabitats is relatively high, and a behavior for reducing this stress would be advantageous. Second, our laboratory investigation of preference for dark-colored vs. light-colored substrata indicates strong directionality toward dark areas of the substratum, potentially a cue for protective cover. Third, mucus trails seem to be of little importance to snails in the formation of aggregations: snails did not follow trails leading to white areas of the substratum. Therefore, cues indicative of protective cover may be of greater importance to exposed snails than cues indicating the presence of other snails. In the absence of protective cover, snails may orient to other snails with the dark shell as a primary cue.

The results of this study show that aggregation is an effective method for reducing desiccation stress due to evaporative water loss. Microhabitat choice also appears to be an effective method, however, and the question as to whether aggregations are formed as a means of reducing desiccation or as a result of crowding into protected microhabitats upon exposure during low tide may have more than one answer. In unprotected microhabitats, snails may actively seek to form aggregations. In protected microhabitats, aggregations may be the result of a common orientation toward cues representing protective cover.

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